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In vitro antifungal activity of some South African medicinal plants

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The acetone extracts of 12 plants used in folkloric medicine in the Eastern Cape of South Africa, were investigated for their *in vitro* antimycotic activity against five fungi using the agar dilution method. The extracts showed significant inhibition of growth of the test organisms at varying concentrations. Extracts from *Arctotis arctotoides* showed the highest activity at concentrations varying from 0.1 to 10mg ml^{-1}, followed by *Usnea barbata,* a lichen, while *Grewia occidentalis* demonstrated the least activity. Extracts from *A. arctotoides, U. barbata, Combretum caffrum, Aloe ferox, Salix capensis, Schotia latifolia* and *Prunus persica* were fungicidal at 10mg ml^{-1} which was the highest concentration tested. The fungi differed significantly in their susceptibility to plant extracts with *Alternaria alternaria,* and *Mucor hiemalis,* being completely inhibited at 5 and 10mg ml^{-1} by most of the extracts.

Introduction

Human mycosis, including infections of the skin and mucosal surfaces, constitute a serious problem in developing countries. Fungal infections have been observed to be the primary cause of mortality in patients with severely impaired immune mechanisms (Kulberg 1997). The number of reported cases of immunocompromised patients which frequently develop opportunistic and superficial mycoses, such as candidiasis and aspergillosis, has increased dramatically in patients with AIDS in the recent years (Portillo *et al*. 2001, Silva *et al*. 2001). Despite several available antimycotic drugs, the treatment of immunocompromised patents is still limited due to a number of factors. Such factors include low drug potency, poor solubility, emergence of resistant strains and drug toxicity (McCutchen *et al.* 1994, Li *et al.* 1995, Nwosu and Okafor 1995). The increase of the AIDS-related fungal opportunistic pathogens and emergence of resistant strains in recent years have lent additional urgency to antifungal studies (Silva *et al*. 2001). In the search for new, safer and more effective antifungal agents, it is important to have new chemotherapeutic approaches for the treatment of patients with common and rare fungal infectious diseases. Extracts and natural products from plants offer a wide variety of bioactive compounds that could meet these requirements (Janssen and Cawenbergh 1990, Shu 1998). In Kenya for instance, water extracts from leaves of *Schizozygia coffaeoides* are used for washing ringworm-infected skin, and root extracts mixed with coconut oil is used for the treatment of skin sores (Omino and Kokwara 1993, Kariba *et al.* 2001). In continuation of our effort to screen extracts from many medicinal plants used by the people of the Eastern Cape, South Africa, against microbial infections (Grierson and Afolayan 1999a), we evaluated the antimycotic activity of 12 medicinal plant species against *Alternaria alternaria, Aspergillus niger, Mucor hiemalis, Penicillium notatum* and *Schizophyllum commune.* We also report the ethnomedical information obtained on these plants from herbalists, traditional healers and the rural dwellers of the province.

Materials and Methods

Ethnomedical information and plant collection

Information on the plant usage presented in this paper was based on literature surveys and data collection procedure as previously reported by Grierson and Afolayan (1999b). Further information was collected through consultations with some practicing Xhosa traditional healers, Inyangas, Sangomas and experienced rural livestock farmers that have been using herbs for the treatment of their animals for years. Plant materials were collected from natural populations and voucher specimens prepared and deposited at the University of Fort Hare herbarium. The botanical names and families, Xhosa names, traditional usage, the parts used and the method of preparation of plant materials are given in Table 1. Although *Prunus persica* and *Tagetes minuta* are not indigenous plants to South Africa, they are cosmopolitan in distribution and their use as medicinal plants is widely reported.

Preparation of extracts

Air-dried plant materials were extracted in acetone by shaking for 30 minutes on an orbital shaker. Acetone is an easier solvent to use when screening a number of plants due to

Table 1: Ethnomedical information on some medicinal plants used in the Eastern Cape, South Africa

Plant species and family	Local name	Parts used	Popular uses	Method of preparation
Aloe ferox Mill. (Asphodelaceae)	ikhala	Leaves	Treatment of gallsickness and redwater diseases in livestock	Infusion administered orally
Arctotis arctotoides (L.F.) O. Hoffm. (Asteraceae)	Ubushwa	Shoot	Treatment of epilepsy. Against sores and wounds Indigestion and catarrh of the stomach	Patients wash with decoction daily Leaf juice or paste applied twice daily Decoction taken orally as frequently as possible
Cheilanthes viridis (Forsk.) (Adiantaceae)	Fern	Fronds	To treat wounds	Dried, powdered and sprinkled Swartz wounds
Combretum caffrum Kuntze (Combretaceaea)	Umdubi	Bark and leaves	Redwater disease and conjunctivitis in livestock	Crush and boil in water or squeeze the juice and apply to the eyes
Grewia occidentalis L. (Tiliaceae)	Umnqabaza, Unvileni, umqaqoba	Twigs and leaves	To treat wounds	Infusion used as a lotion on wounds
Malva parvifolia L. (Malvaceae)	Umnyamathi	Shoot	Treatment of wounds	Macerated with or without heated brown sugar
Polystichum pungens Kaulf (Aspidiaceae)	Fern	Fronds	Treatment of wounds	Dried and powdered, sprinkled on wounds
Prunus persica L. (Rosaceae)	Pesika	Roots	Treatment of gonorrhoea	Roots are boiled in water and patient drinks three times daily
Salix capensis Thunb. (Salicaceae)	umgcunube	Bark and leaves	Treatment of redwater and gallsickness in animals and expulsion of retained placenta after birth	Decoction given orally to the animal
Schotia latifolia Jacq. (Fabaceae)	umGxam	Bark and leaves	Redwater disease in livestock	Decoction administered orally
Tagetes minuta L. (Asteraceae)	Nukanuka	Shoot	Against syphilis	Ground into powder and the paste applied directly to the sore
Usnea barbata Web. (Usneaceae)	Old man's beard, Beard moss or tree moss	Whole lichen	Treatment of mammary infections in cattle	Affected organ is washed several times with decoction of plant material
			Indigestion and catarrh of the stomach of man	Tincture or decoction taken orally several times daily

its volatility, miscibility with polar and non-polar solvents and its relatively low toxicity to the test organisms (Eloff 1998). The extracts were filtered using a Buchner funnel and Whatman filter paper No. 1, and the filtrates taken to dryness with a vacuum evaporator at 40°C. Each extract was suspended in acetone to yield 50mg residue ml^{-1} solvent.

Antifungal testing

Test fungi, *Alternaria alternaria, Aspergillus niger, Mucor hiemalis, Penicillium notatum* and *Schizophyllum commune,* were obtained from the Department of Biochemistry and Microbiology of Rhodes University in Grahamstown. Each culture was maintained on potato dextrose agar (PDA) and was recovered for testing by subculturing on fresh PDA for three days.

Agar plates were prepared in the usual fashion by autoclaving before the addition of the extracts. Each extract was filtered through sterile 0.22mm syringe-fitted filters to remove possible microbial contaminants, before mixing with the molten agar (at 45°C) to final concentrations of 10, 5, 1, 0.5 and 0.1mg extract residue ml^{-1} molten agar and poured into the Petri dishes. Blank plates containing only PDA or 2% acetone served as controls (Afolayan and Meyer 1997). The prepared plates containing the extracts were inoculated with plugs obtained from the actively growing margin of the fungi plates and incubated at 25°C for five days. Diameter of fungal growth was measured and expressed as means of percentage growth inhibition of three replicates. Significance differences within the means of the treatments and the controls were calculated using the LSD statistical test (Steel and Torrie 1960).

Results and Discussion

The summary of the ethnomedical data of the 12 medicinal plants studied are presented in Table 1. The species belong to different families with the exception of *Arctotis arctotoides* and *Tagetes minuta* which both belong to the Asteraceae family. Each of the plants is widely used for medicinal purposes throughout the Eastern Cape for the treatment of humans or livestock.

The results of the antifungal assay, arranged in order of importance in antifungal activity, are presented in Table 2. The majority of the extracts showed significant antimycotic activity against the test organisms. A careful study of the results revealed that extract from *Arctotis arctotoides* caused the highest growth inhibition in most of the fungi, with 100% inhibition in *Alternaria alternaria, Aspergillus niger, Mucor hiemalis* and *Schizophyllum commune* at concentrations of 5 and 10mg ml^{-1}. Fifteen of the treatments with *A. arctotoides*

Table 2: Antifungal activity of acetone extracts of some medicinal plants used in the Eastern Cape, South Africa

Plant species	Parts used	Conc. (mg ml^{-1})	Growth inhibition (%)				
			A. alternaria	*A. niger*	*M. hiemalis*	*P. notatum*	*S. commune*
Arctotis arctotoides	Shoot	10	100.00^{e}	100.00^{d}	100.00^{d}	91.47^{f}	100.00^{e}
		5	100.00^{e}	62.28^{c}	100.00^{d}	82.30^{e}	100.00^{e}
		1	79.74^{d}	22.81^{b}	58.06^{c}	47.71^{d}	60.75^{d}
		0.5	55.06^{c}	21.56^{b}	14.52^{b}	32.26^{c}	52.50^{c}
		0.1	24.47^{b}	6.30^{a}	0.54^{a}	13.81^{b}	27.42^{b}
		Acetone	1.32^{a}	-0.93^{a}	0.00^{a}	0.00^{a}	0.00^{a}
		Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
		LC50 (mg ml^{-1})	0.43	3.76	0.91	1.26	0.35
Usnea barbata	Shoot	10	100.00^{f}	61.57^{e}	78.47^{d}	67.13^{e}	51.60^{e}
		5	76.70^{e}	56.27^{e}	75.27^{d}	60.13^{d}	50.53^{de}
		1	57.93^{d}	27.80^{d}	46.80^{c}	43.77^{c}	42.47^{d}
		0.5	48.93^{c}	17.13^{c}	30.10^{b}	39.03^{c}	31.17^{c}
		0.1	39.26^{b}	10.43^{bc}	3.73^{a}	21.07^{b}	13.47^{b}
		Acetone	6.27^{a}	3.90^{ab}	0.00^{a}	-1.56^{a}	0.00^{a}
		Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
		LC50 (mg ml^{-1})	0.56	4.12	1.45	2.52	4.74
Combretum caffrum	Bark	10	100.00^{f}	58.30^{e}	100.00^{f}	49.30^{d}	100.00^{d}
		5	62.00^{e}	29.00^{d}	92.30^{e}	36.30^{c}	73.30^{c}
		1	18.00^{d}	24.00^{cd}	39.00^{d}	9.00^{b}	25.30^{b}
		0.5	12.00^{c}	16.30^{cb}	20.30^{c}	4.00^{a}	-6.00^{a}
		0.1	2.50^{ab}	12.00^{b}	6.30^{b}	2.30^{a}	-1.70^{a}
		Acetone	5.00^{b}	2.50^{a}	0.00^{a}	0.00^{a}	1.30^{a}
		Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
		LC50 (mg ml^{-1})	3.91	8.58	1.83	>10	3.06
Aloe ferox	Leaf	10	100.00^{e}	37.87^{ff}	64.50^{d}	50.73^{f}	55.47^{d}
		5	100.00^{e}	29.20^{e}	49.47^{c}	43.30^{e}	28.47^{c}
		1	18.87^{d}	15.50^{dc}	2.13^{b}	26.80^{d}	9.43^{b}
		0.5	17.00^{dc}	17.80^{d}	0.00^{a}	20.87^{c}	8.70^{b}
		0.1	10.97^{bc}	9.60^{bc}	0.00^{a}	10.40^{b}	-5.73^{a}
		Acetone	5.00^{ab}	3.13^{ab}	0.00^{a}	8.23^{b}	6.77^{b}
		Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
		LC50 (mg ml^{-1})	2.53	>10	5.18	9.51	8.99
Salix capensis	Bark	10	100.00^{d}	15.00^{b}	100.00^{f}	78.00^{b}	57.00^{d}
		5	100.00^{d}	6.67^{a}	79.00^{e}	5.67^{a}	27.00^{c}
		1	10.33^{c}	5.00^{a}	24.00^{d}	3.00^{a}	2.33^{ab}
		0.5	4.67^{b}	6.33^{a}	7.00^{c}	0.00^{a}	4.00^{ab}
		0.1	3.67^{b}	1.00^{a}	2.67^{b}	1.33^{a}	5.67^{b}
		Acetone	1.32^{a}	4.00^{a}	1.32^{b}	1.00^{a}	1.50^{ab}
		Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
		LC50 (mg ml^{-1})	2.77	>10	2.89	8.06	8.33
Schotia latifolia	Bark	10	69.33^{f}	12.67^{b}	100^{c}	10.33^{d}	61.67^{d}
		5	51.00^{e}	5.67^{ab}	83.33^{d}	7.67^{cd}	24.00^{c}
		1	23.33^{d}	7.00^{ab}	40.33^{c}	3.00^{abc}	8.33^{ab}
		0.5	14.67^{c}	8.33^{ab}	18.67^{b}	1.33^{ab}	7.67^{ab}
		0.1	1.00^{ab}	6.67^{ab}	0.00^{a}	6.00^{bcd}	15.67^{bc}
		Acetone	1.80^{a}	2.10^{a}	0.00^{a}	1.50^{ab}	3.67^{ab}
		Control	0.00	0.00	0.00	0.00	0.00
		LC50 (mg ml^{-1})	4.86	>10	1.90	>10	8.45
Prunus persica	Root	10	58.33^{c}	33.67^{c}	100.00^{d}	-12.23^{a}	36.77^{d}
		5	35.67^{b}	28.33^{bc}	100.00^{d}	4.50^{b}	11.40^{c}
		1	19.93^{ab}	22.60^{bc}	32.53^{c}	0.87^{b}	8.17^{bc}
		0.5	9.07^{a}	22.17^{bc}	13.43^{b}	3.43^{b}	3.23^{ab}
		0.1	6.40^{a}	20.53^{b}	0.00^{a}	0.13^{b}	2.17^{ab}
		Acetone	0.00^{a}	18.67^{b}	0.00^{a}	5.10^{b}	2.07^{ab}
		Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{b}	0.00^{a}
		LC50 (mg ml^{-1})	8.16	>10	2.04	>10	>10

Table 2 cont.

Plant species	Parts used	Conc. (mg ml^{-1})	Growth inhibition (%)				
			A. alternaria	*A. niger*	*M. hiemalis*	*P. notatum*	*S. commune*
Tagetes minuta	Shoot	10	39.97^{f}	20.87^{c}	27.93^{c}	60.93^{e}	48.23^{e}
		5	33.00^{e}	19.9^{c}	17.73^{b}	49.87^{d}	39.67^{d}
		1	13.00^{d}	22.67^{c}	0.00^{a}	17.17^{c}	11.47^{c}
		0.5	8.95^{c}	20.83^{c}	0.00^{a}	9.78^{bc}	8.07^{c}
		0.1	4.79^{b}	22.67^{c}	0.00^{a}	3.67^{ab}	7.47^{bc}
		Acetone	0.97^{a}	9.10^{b}	0.00^{a}	16.97^{c}	2.25^{ab}
		Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
		LC50 (mg ml^{-1})	>10	>10	>10	5.06	>10
Polystichum pungens	Fronds	10	56.15^{d}	15.30^{b}	0.00^{a}	29.90^{d}	0.00^{a}
		5	37.69^{c}	13.01^{b}	0.00^{a}	13.98^{dc}	0.00$_{a}$
		1	13.33^{b}	16.27^{b}	0.00^{a}	-5.34^{ab}	0.00^{a}
		0.5	0.00^{a}	12.13^{b}	0.00^{a}	-10.87^{ab}	0.00^{a}
		0.1	0.00^{a}	13.02^{b}	0.00^{a}	-22.95^{a}	0.00^{a}
		Acetone	0.00^{a}	0.01^{a}	0.00^{a}	1.713^{bc}	0.00^{a}
		Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{bc}	0.00^{a}
		LC50 (mg ml^{-1})	8.33	>10	>10	>10	>10
Malva parvifolia	Leaves	10	26.78^{c}	18.39^{c}	0.00^{a}	2.14^{d}	27.18^{d}
	and	5	17.25^{b}	22.40^{c}	0.00^{a}	7.08^{e}	24.62^{c}
	stems	1	0.80^{a}	20.01^{c}	0.00^{a}	-3.80^{ab}	18.97^{b}
		0.5	1.53^{a}	17.98^{c}	0.00^{a}	-2.72^{abc}	0.00^{a}
		0.1	-0.43^{a}	16.37^{cb}	0.00^{a}	-5.97^{a}	0.00^{a}
		Acetone	3.09^{a}	11.03^{b}	0.00^{a}	1.07^{cd}	0.00^{a}
		Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{bcd}	0.00^{a}
		LC50 (mg ml^{-1})	>10	>10	>10	>10	>10
Cheilanthes viridis	Fronds	10	11.80^{c}	26.38^{e}	0.00^{a}	-0.55^{ab}	15.38^{b}
		5	14.13^{c}	22.81^{de}	0.00^{a}	-2.18^{a}	14.87^{b}
		1	5.89^{b}	15.59^{cbd}	0.00^{a}	-1.11^{a}	0.00^{a}
		0.5	3.59^{ab}	14.40^{cb}	0.00^{a}	4.35^{b}	0.00^{a}
		0.1	4.34^{ab}	19.26^{cde}	0.00^{a}	1.08^{ab}	0.00^{a}
		Acetone	3.09^{ab}	11.03^{b}	0.00^{a}	1.07^{ab}	0.00^{a}
		Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{ab}	0.00^{a}
		LC50 (mg ml^{-1})	>10	>10	>10	>10	>10
Grewia occidentalis	Leaves	10	24.39^{c}	17.57^{cb}	0.00^{a}	1.08^{b}	0.00^{a}
	and	5	16.55^{b}	16.33^{cb}	0.00^{a}	-8.15^{a}	0.00^{a}
	small	1	-0.84^{a}	19.22^{c}	0.00^{a}	-10.34^{a}	0.00^{a}
	twigs	0.5	-0.42^{a}	22.36^{c}	0.00^{a}	-7.63^{a}	0.00^{a}
		0.1	2.28^{a}	18.03^{cb}	0.00^{a}	-8.15^{a}	0.00^{a}
		Acetone	3.09^{a}	11.03^{b}	0.00^{a}	1.07^{b}	0.00^{a}
		Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{b}	0.00^{a}
		LC50 (mg ml^{-1})	>10	>10	>10	>10	>10

Values are means of percentage growth inhibition of three replicates; values within a column followed by the same superscript of the same plant species are not significantly different at P<0.05 according to the LSD test. LC50 values were calculated by extrapolation

had more than 50% inhibition against all the fungi at all concentrations. The genus *Arctotis* is native mainly to southern Africa, although El Dahmy *et al*. (1986) reported its occurrence in Egypt. The distribution of *A. arctotoides* in South Africa ranges from south-west Cape through the southern and eastern provinces to KwaZulu-Natal where it occurs up to 2 745meters above sea level, forming carpets on marshy stream banks in drainage ditches or at higher altitudes on damp environments (Hilliard 1977). The herb is reported to be used in large quantities for the treatment of epilepsy, indigestion and catarrh of the stomach. The Xhosas apply the leaf juice or paste for the treatment of wounds.

Only one test organism, *Alternaria alternaria,* was 100% inhibited by the *Usnea barbata* extract. A further 10 treatments of this extract inhibited the organisms by more than 50% (Table 2). *U. barbata* is a fruticose lichen found on trees in damp forests of Hogsback in the Eastern Cape. It has a recorded history of therapeutic use dating back over 3 000 years in Chinese medicine (Tilford 1997). In the Eastern Cape province, the lichen is used by dairy farmers to treat mammary infections in cows, however, its medicinal importance for humans has not been recorded.

In this study, the third most active extract was observed in *Combretum caffrum* where eight treatments showed more

than 50% fungal inhibition. *C. caffrum* is a tree that grows in moist areas of the Eastern Cape. The medicinal importance of this species was established when the anti-cancer compounds, stibenes and bibenzyls, were isolated from its heartwood (Brrokes *et al.* 1999).

In this study, extracts from eight plants showed more than 50% inhibition of the test fungi. Generally, *Polystichum pungens, Malva parvifolia, Cheilanthes viridis* and *Grewia occidentalis* showed the least antifungal activity (Table 2). While *M. parvifolia* and *G. occidentalis* are angiosperms, *P. pungens* and *C. viridis* are pteridophytes. These plants are the four commonest species used for wound treatment in the Eastern Cape. A previous study has reported the antibacterial activity of *G. occidentalis, P. pungens* and *C. viridis* against Gram positive and Gram negative bacteria, indicating a broad spectrum antibacterial property (Grierson and Afolayan 1999a) while extracts of *M. parvifolia* were not active against any of the organisms tested. Shale *et al.* (1999), however, report positive antibacterial activity of the hexane and methanol extracts of the roots of this plant, but noted poor activity of the methanol leaf-extract. Further work may be necessary to justify the use of *M. parvifolia* by the traditional healers for the treatment of wounds. This plant is, however, sometimes used with heated brown sugar, which may be the reason for its activity and thus justify its use (Grierson and Afolayan 1999a). Herbal remedies used by the people of the Eastern Cape often comprise several plants that belong to different taxonomic groups. It is, therefore, difficult to know the precise contribution of every plant to the property of a given remedy mixture. Nonetheless, most of the selected plants showed interesting antimicrobial properties. Specifically, 10 of the plants exhibited selective antimycotic activity to varying degrees, while seven of the species, *A. arctotoides, U. barbata, C. caffrum, A. ferox, S. capensis, S. latifolia* and *P. persica* showed fungicidal activity (100% inhibition of growth) at 10mg ml^{-1} which was the highest concentration tested in the study. It is interesting to note some correlation between the claims of traditional healers and the demonstrated antimicrobial activity of the plants. The susceptibility of *Aspergillus niger* to the extract of *A. arctotoides* (100% growth inhibition) is particularly noteworthy, as the fungus has recently been implicated in cases of immunocompromised patients that frequently develop opportunistic and superficial mycosis (Ngane *et al*. 2000, Portillo *et al*. 2001, Silva *et al*. 2001). It is worth noting that two fungal species, namely *Mucor hiemalis* and *Schizophyllum commune* appear to be more resistant to extracts of plants tested in this study. These findings may provide leads in the ongoing search for novel antimycotic agents. It is also noteworthy that in a few cases plant extracts apparently stimulated the growth of some of the test organisms (negative inhibition percentages observed in Table 2). As crude extracts were used, stimulatory substances present might have overshadowed the effect of the antifungal agents.

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References

Afolayan AJ, Meyer JJM (1997) The antimicrobial activity of 3,5,7–trihydroxyflavone isolated from the shoots of *Helichrysum aureonitens*. ***Journal of Ethnopharmacology* 57**: 177–181

Brrokes KB, Doudoukina OV, Katsoulis LC, Veale DJH (1999) Uteroactive constituents from *Combretum kraussii.* ***South African Journal of Chemistry* 52**: 127–133

El Dahmy S, Sarg T, Salem SA, Jakupovic J, Bohlmann F (1986) New guaianolides and farnesol derivates from *Arctotis arctotoides*. ***Planta Medica* 52**: 365–367

Eloff JN (1998) Which extractant should be used for the screening and isolation of antimicrobial compounds from plants? ***Journal of Ethnopharmacology* 60**: 1–8

Grierson DS, Afolayan AJ (1999a) Antibacterial activity of some indigenous plants used for the treatment of wounds in the Eastern Cape, South Africa. ***Journal of Ethnopharmacology* 66**: 103–106

Grierson DS, Afolayan AJ (1999b) An ethnobotanical study of plants used for the treatment of wounds in the Eastern Cape, South Africa. ***Journal of Ethnopharmacology* 66**: 327–332

Hilliard OM (1977) Compositae in Natal. University of Natal Press, Pietermaritzburg, South Africa, pp 536–537

Janssen PA, Cawenbergh G (1990) Antifungal therapy of the future. In: Jacobs PH, Nall L (eds) Antifungal Drug Therapy. New York, USA, Marcel Dekker

Kariba RM, Siboe GM, Dossaji SF (2001) *In vitro* antifungal activity of *Schizozygia coffaeoides* Bail. (Apocynaceae) extracts. ***Journal of Ethnopharmacology* 74**: 41–44

Kulberg BJ (1997) Trends in immunotherapy of fungal infections. ***European Journal of Clinical Microbiology and Infectious Diseases* 16**: 51–55

Li E, Clark AM, Hufford CD (1995) Antifungal evaluation of pseudolaric acid B, a major constituent of *Pseudolarix kaempferi.* ***Journal of Natural Products* 58**: 57–67

McCutchen AR, Ellis SM, Hancock REW, Towers GHN (1994) Antifungal screening of medicinal plants of British Columbian native peoples. ***Journal of Ethnopharmacology* 44**: 157–169

Ngane AN, Biyiti L, Zollo PHA, Bouchet PH (2000) Evaluation of antifungal activity of extracts of two Cameroonian Rutaceae: *Zanthoxylum leprieurii* Guill et Perr. And *Zanthoxylum xanthoxyloides* Waterm. ***Journal of Ethnopharmacology* 70**: 335–342

Nwosu MO, Okafor JI (1995) Antifungal activity of plant extracts. ***Mycoses* 38**: 191–195

Omino EA, Kokwaro JO (1993) Ethnobotany of Apocynaceae species in Kenya. ***Journal of Ethnopharmacology* 40**: 167–180

Portillo A, Vila R, Freixa B, Adzet T, Canigueral S (2001) Antifungal activity of Paraguayan plants used in traditional medicine. ***Journal of Ethnopharmacology* 76**: 93–98

Shale TL, Stirk WA, van Staden J (1999) Screening of medicinal plants used in Lesotho for anti-bacterial and anti-inflammatory activity. ***Journal of Ethnopharmacology* 67**: 347–354

Shu YZ (1998) Recent natural products based drug development: a pharmaceutical industry perspective. ***Journal of Natural Products* 61**: 1053–1071

Silva MV, Costa TR, Costa MR, Ferreira EC, Fernandes OFL, Santos SC, Liao LM, Ferri PH, Paula JR, Ferreira HD, Silva MRR (2001) Growth inhibition effect of Brazilian Cerrado plant extracts on *Candida* species. ***Pharmaceutical Biology* 39**: 138–141

Steel RGD, Torrie JH (1960) Principles and Procedures of Statistics. Mc Graw-Hill, New York, USA

Tilford GL (1997) Edible and medicinal plants of the west. In: Missoula MT (ed.) Mountain Press, pp 148–149